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Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris*) genotypes

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Abstract Ten snap bean (*Phaseolus vulgaris*) genotypes were screened for polymorphism with 400 RAPD (random amplified polymorphic DNA) primers. Polymorphic RAPDs were scored and classified into three categories based on ethidium bromide staining intensity. An average of 5.19 RAPD bands were scored per primer for the 364 primers that gave scorable amplification products. An average of 2.15 polymorphic RAPDs were detected per primer. The results show that primer screening may reduce the number of RAPD reactions required for the analysis of genetic relationships among snap-bean genotypes by over 60%. Based on the analysis of the distribution of RAPD amplification, the same number of polymorphic RAPDs were amplified from different genotypes for all RAPD band intensity levels. A comparison of RAPD band amplification frequency among genotypes for the three categories of bands classified by amplification strength revealed a measurable difference in the frequencies of RAPDs classified as faint (weakly amplifying) compared to RAPD bands classified as bold (strongly amplifying) indicating a possible scoring error due to the underscoring of faint bands. Correlation analysis showed that RAPD bands amplified by the same primer are not more closely correlated then RAPD bands amplified by different primers but are more highly correlated then expected by chance. Pairwise comparisons of RAPD bands indicate that the distribution of RAPD amplification among genotypes will be a useful criterion for establishing RAPD band identity. For the average pairwise comparison of genotypes, 50% of primers tested and 15.8% of all scored RAPDs detected polymorphism. Based on RAPD data Nei's average gene diversity at a locus was 0.158 based on all scorable RAPD bands and 0.388 if only polymorphic RAPD loci were considered. RAPD-derived relationships among genotypes are reported for the ten genotypes included in this study. The data presented here demonstrate that many informative, polymorphic RAPDs can be found among snap bean cultivars. These RAPDs may be useful for the unique identification of bean varieties, the organization of bean germplasm, and applications of molecular markers to bean breeding.

Key words *Phaseolus vulgaris* · Snap bean
RAPD variation

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Introduction

Common bean (*Phaseolus vulgaris* L.) market classes are defined by seed and pod characteristics and can be broadly classified into dry beans, grown for the mature seed, and snap beans which are grown for the edible pods. Phaseolin protein electrophoretic patterns have been useful for the identification of centers of domestication (Gepts et al. 1986), races (Singh et al. 1991a), and genetic diversity in the common bean (Koenig et al. 1990; Gepts 1990). Allozyme electrophoretic patterns have been evaluated for the characterization of cultivar identity (Adriaanse et al. 1969; Weeden 1984), genetic relationships among cultivars (Bassiri and Adams 1978), and genetic diversity (Bassiri and Adams 1978; Singh et al. 1991b). Studies of allozyme variability in bean have also contributed to the identification of common bean races (Singh et al. 1991a) and centers of domestication (Singh et al. 1991b). However, although useful for understanding the organization of bean germplasm, allozyme and seed protein markers do not discriminate all cultivars or landraces studied. For example, Weeden (1984) was able to establish unique allozyme fingerprints for only 58 out of 90 white seeded snap bean cultivars. In comparison to allozyme polymorphism, RFLP (restriction fragment length polymorphism) variation is relatively more abundant in common bean (Nodari et al. 1992) and could be used

for germplasm studies as well as fingerprinting cultivars. However, in common bean, RFLPs have been used primarily for the construction of genetic linkage maps (Gepts et al. 1993).

The construction of genetic linkage maps in *P. vulgaris* may be easier using RAPD (Williams et al. 1990) genetic markers. Moreover, large-scale studies of genetic relationships among *P. vulgaris* cultivars are now possible, using RAPD data in which all cultivars are distinguishable (Skroch et al. 1992 a, b). The RAPD technique has also allowed the efficient discovery of molecular genetic markers linked to specific genes and has been proposed as a tool for pyramiding disease resistance genes in bean (Kelly et al. 1993; Miklas et al. 1993). Other studies have documented the usefulness of RAPDs in a variety of species, for example, in wheat (Vierling et al. 1992), barley (Tinker et al. 1993), papaya (Stiles et al. 1993), *Brassica oleracea* (Dos Santos et al. 1994), and celery (Yang and Quiros 1993).

However, underscoring, or scoring of false negatives, has been suggested by the evaluation of RAPD data from segregating populations (Weeden et al. 1992; Kennard et al. 1994). The underscoring of RAPDs may be due to competition effects (Heun and Helentjaris 1993) and has been correlated with RAPD band intensity (Weeden et al. 1992).

An additional concern about RAPD data has been the communication and the reproducibility of results across laboratories (Penner et al. 1993). To facilitate communication among labs, RAPD markers might be "fingerprinted" by the distribution of RAPD amplification among a set of standard genotypes. The efficiency of this strategy for establishing RAPD marker identity will be influenced by the independence of RAPD polymorphisms relative to each other and the distribution of polymorphisms across genotypes. The independence and frequency of genetic markers in the relevant germplasm will also influence their efficiency for application to genotype identification, germplasm analysis and marker assisted breeding.

In this paper we report the results of a survey of 400 RAPD primers for polymorphism among a set of ten snap bean genotypes. Data from such a primer screen serves a number of objectives. The first objective was the evaluation of primer pre-screening as a strategy for reducing the costs of germplasm studies involving large numbers of genotypes. Depending on the variability among RAPD primers for the number of RAPDs detected, the accomplishment of this objective would result in the discovery of primers that generate relatively large numbers of RAPDs. The second objective was the quantification of RAPD amplification frequency and an analysis of the relative independence of RAPDs among snap bean genotypes. This was to provide information germane to the establishment of the identity of individual RAPD bands as well as to the efficiency of the application of RAPDs as molecular genetic markers in the snap bean germplasm. The third objective was the evaluation of potential underscoring of RAPDs through a comparison of the amplification frequencies of RAPDs of differing amplification strength. The fourth objective was to obtain estimates of snap bean genetic diversity and genetic relationships among genotypes based on RAPD marker data.

Materials and methods

Plant material

Nine snap bean cultivars and one snap bean breeding line were chosen for primer screening. These lines vary for an array of phenotypic traits including seed color, pod color, pod length and various disease resistances (Table 1) as well as seed size and shape (data not shown). In addition to the evident phenotypic variability the development of these lines can be credited to at least nine different breeding programs (Table 1). These ten phenotypically distinguishable snap bean lines, with diverse breeding histories, were selected to obtain a broad sample of the genetic diversity available in the snap bean germplasm.

Table 1 Description[a] of snap bean genotypes used for screening RAPD primers

Name	Pod length (cm)	Pod color	Seed color	Disease resistances[b]	Breeding program
Dandy	10	Green	White	BCMV, BCMV-15, ANTH	Rogers Brothers Seed Co.
Duchess	12.7–14	Green	Brown	BCMV-15	Musser Seed Co.
Epoch	9–10	Green	White	CT, BCMV, BCMV-15, BBS	Wilber Ellis Seed Co.
Espada	15	Green	White	BCMV, BCMV-15, HB, ANTH	Harris Moran Seed Co.
Flo	14.5	Green	White	BCMV, ANTH	Asgrow Seed Co.
FM456	15	Green	White	BCMV-15	Ferry Morse Seed Co.
G6–6	–	Green	White	–	University of Wisconsin
Hystyle	12.7	Green	White/green	BCMV, BCMV-15, CT	Harris Moran Seed Co.
Keygold	15.2	Yellow	White	BCMV, BCMV-15	Keystone
Kinghorn wax	15.2	Yellow	White	BCMV	F. H. Woodruff Seeds

[a] As described by seed companies
[b] BCMV-15 = Common bean mosaic virus, New York strain 15; BCMV = Common bean mosaic virus; HB = Halo Blight (race 2); CT = Curly top; ANTH = Anthracnose; and BBS = Bacterial Brown Spot

DNA extraction

Plants were grown under continuous light at approximately 24 °C. DNA was extracted from immature unifoliate leaves 6–8 days after planting. Tissue was harvested and bulked from 4–8 plants of each genotype. Approximately 1.0–1.5 g of fresh leaf tissue was ground in 10 ml of extraction buffer at 65 °C using a mortar and pestle. The extraction buffer contained 2% CTAB, 100 mM TRIS (pH = 8.0) 20 mM EDTA (pH = 8.0) 1.4 M NaCl, and 1% PVP (polyvinylpyrolidone) (2 x extraction buffer from Rogers and Bendich 1988). After grinding, tissue was transferred to centrifuge tubes and allowed to incubate for 30 min in a water bath at 65 °C. The mixture was then extracted once with an equal volume of 24:1 chloroform and isoamyl alcohol.

After organic and aqueous phases of the extraction mixture were separated by centrifugation, nucleic acids were precipitated by pipetting the aqueous phase into at least 3 vol of a 6:1 mixture of 95% ethanol and 7.5 M ammonium acetate. Precipitating nucleic acids were allowed to stand overnight at − 20 °C. Floating precipitate was transferred with a wide-bore pipette into 1.5-ml micro-centrifuge tubes. After spinning for 15–30 s at high speed in a micro centrifuge, alcohol was poured off and pellets were rinsed with a 70% ethanol solution and re-pelleted. The pellet was re-hydrated in a dilute TE buffer (1 mM Tris, pH = 8.0, and 0.1 mM EDTA, pH = 8.0). RNA was removed by treatment with RNase A at 100 µg/ml for 1 h at 37 °C. Samples were then spun to remove any remaining debris and transferred to clean micro-centrifuge tubes. DNA was precipitated by the addition of at least 2 vol of a 20:1 mixture of 95% ethanol and 3 M sodium acetate. After precipitation and pelleting, DNA was re-hydrated in TE buffer (1 mM Tris, pH = 8.0, and 0.1 mM EDTA, pH = 8.0) and quantified using a Hoeffer Scientific TKO 100 Fluorometer.

RAPD reactions

Four-hundred RAPD primers, from Operon primer kits A, B, and F through Z, were obtained from Operon Technologies Inc., Alameda, Calif. RAPD reactions were performed in an Idaho Technology, Air Thermal Cycler, model 1605, in thin-walled glass capillary tubes. Cycling temperature settings were 91 °C for denaturation, 42 °C for annealing, and 72 °C for elongation. In the first two cycles, cycling times were 1 min for denaturation, 7 s for annealing, and 70 s for elongation. During the subsequent 38 cycles denaturation was set for 1 s, annealing for 7 s and elongation for 70 s. These 40 cycles were followed by a 4-min hold at 72 °C. The reaction buffer was composed of 50 mM Tris, pH 8.5, 2 mM $MgCl_2$, 20 mM KCl, 500 µg/ml BSA, 2.5% Ficoll 400, and 0.02% (w/v) xylene cyanol. Reactant concentrations were 100 µM dNTPs, 2 ng/µl of DNA template, 0.4 µM of RAPD primer, and 0.6 unit of *Taq* DNA polymerase in a final reaction volume of 10 µl.

RAPD products were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide. Subsequently, gels were placed on a UV light trans-illuminator and results recorded on Polaroid 667 film. In the following discussion of methods and results the term "RAPD band" will refer to a set of co-migrating RAPD fragments amplified by the same primer over a set of genotypes. For each RAPD band amplification was scored for individual genotypes as "1" for presence and "0" for absence. Only RAPDs which showed consistent and discreet differences for RAPD fragment amplification among genotypes were scored. Intensity differences were scored as polymorphic in cases where such differences could be scored unambiguously. If RAPDs could not be scored unambiguously for a particular genotype, then the score for that genotype was entered as missing data. If RAPD amplification appeared to be significantly reduced in a particular genotype for all RAPDs or RAPDs in a particular size range, then the corresponding RAPD fragment scores for that genotype were entered as missing data.

RAPD bands were also rated as faint (3), medium (2), or bold (1) based on relative band intensity (Fig. 1). The authors admit that scoring criteria were only subjectively enforced. However, data for 50 of the primers used in this study were replicated (Skroch and Nienhuis 1995). The linear correlation of band classification into these three

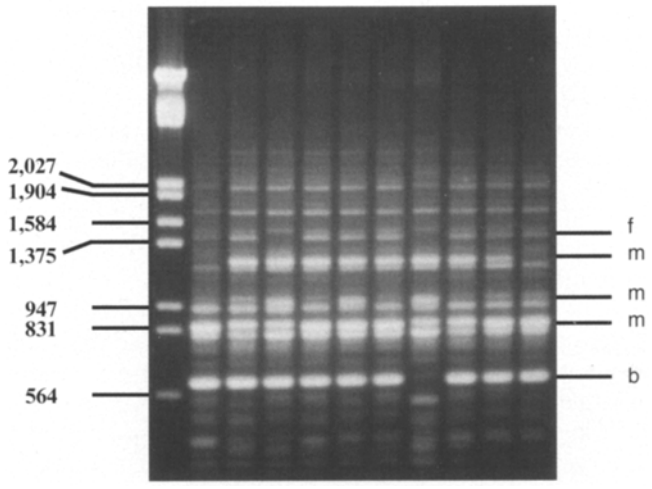


Fig. 1 Amplification products for the ten snap-bean genotypes generated by RAPD amplification using Operon primer P1. The numbers to the left of the picture designate the sizes in base pairs of the Lamda DNA/*Hin*dIII + *Eco*R1 DNA size markers. The letters *f*, *m*, or *b* at the right of the picture indicate the classification of each of the polymorphic bands into faint, medium, or bold categories

categories for the data reported here with replicated data was 0.66 ($P < 0.01$), indicating that band classifications represent real differences in amplification strength.

Data analysis

The hypothesis that polymorphic RAPD band amplification was uniformly distributed among genotypes was tested using a chi-square test. The number of RAPDs amplified per genotype averaged over all ten genotypes was used as the expectation for each test. Individual and total chi-square tests were performed for the set of all RAPD bands and for each class (faint, medium, or bold) separately.

The frequency of amplification among the ten genotypes in this study was tabulated for polymorphic RAPD markers with no missing data, for the entire data set, and separately for each subset of RAPD bands classified by band amplification strength. Means for each set of RAPD bands classified by amplification strength were calculated and compared using t-tests ($\alpha = 0.05$).

Spearman rank correlation coefficients were calculated for RAPD band pairs for RAPDs amplified by the same or different primers. This analysis included 944 of the 952 pairwise comparisons of RAPD bands amplified by the same primer. Correlations for eight of the possible comparisons could not be computed due to missing data. For comparisons of bands amplified by different primers, 944 pairwise band comparisons were randomly sampled from the 306, 936 possible comparisons among the 784 polymorphic RAPD bands detected in this study. Correlations expected under the assumption of a random association of bands were calculated after band scores had been randomized among genotypes. Thus, four sets of rank correlations of RAPD bands were computed, including correlations for band pairs amplified by the same primer for both observed and randomized data as well as correlations computed for bands amplified by different primers for both observed and randomized data. The magnitudes of these correlations were then used in the computation of means and standard errors. All pairwise comparisons of means were then performed using t tests with $\alpha = 0.05$.

As an alternative measure of band correlation, the number of genotypes for which band scores differed was tabulated for all 952 possible comparisons of RAPD band pairs amplified by the same primer and 952 band pairs randomly selected from all possible pairwise band comparisons. In this study the scores for pairs of RAPD bands may differ for 0–10 genotypes with 0 differences corresponding to a rank correlation of 1 and 10 differences corre-

sponding to a rank correlation of -1. Band comparisons were classified based on the number of differences found and t tests were performed ($\alpha = 0.05$) to test the significance of the difference between the four data sets for proportions of observations in each class. The advantage of this analysis over rank correlation analysis is that the results were in the form of a discrete distribution which could be interpreted by class to pinpoint local tends in the data.

The number of occurrences of the most common amplification state, or putative RAPD allele, was used as a measure of informativeness. For a given band for which the frequency of amplification of the most common RAPD allele is p among N genotypes, $Np[N(1-p)]$ combinations of genotypes will differ for this RAPD. Since there are $N(N-1)/2$ possible pairwise combinations of genotypes, it follows that the proportion of possible crosses for which this band will segregate is $2p(1-p)N/(N-1)$. This formula, is equivalent to Nei's gene diversity at a locus (Nei 1987) for two alleles. Genetic diversity among the genotypes in this study was also estimated using Nei's average gene diversity at a locus, $H[H = \sum h_i n_i/(n_i - 1)r$, where r is the number of loci, n_i is the number of genotype observations at the ith locus, and h_i is one minus the sum of the squared allele frequencies at the ith locus].

Genetic distances were computed for each pairwise comparison of genotypes as the complement to the simple matching coefficient (Gower 1972). Genetic relationships among genotypes were visualized in a dendogram based on the unweighted pair-group method of arithmetic averages (UPGMA). UPGMA analysis was performed using the NTSYS program (Applied Biostatistics).

Random sampling, calculation of rank correlations, and calculation of pairwise band differences were performed using computer programs written in "C" by the first author. Means and standard errors for distributions of correlations were computed using JMP software (SAS institute, Cary, N. C.).

Results and discussion

Primer screening

Of the 400 primers used in this study, 296 or about 75% detected one or more RAPD, 68 showed amplification without detection of polymorphism, while 36 gave weak or no amplification. The 364 primers showing good amplification generated from 1 to 13 scorable amplification products with a mean of 5.19 for a total of 1894 RAPD bands tested (Fig. 2a). The number of polymorphic bands scored for each of the 364 primers (Table 2) ranged from 0 to 7 with a mean of 2.15 (Fig. 2b) for a total of 784 polymorphic RAPD bands scored. Based on these results, the generation of 100 RAPDs among these ten snap bean genotypes would require only the 17 primers J4, O13, R7, A2, A7, V10, X11, G8, M12, U19, O19, U15, F13, J5, W6, T15 and O5. However, on average, a random selection would require data from 46.5 primers. Thus, we were able to identify a set of primers that could be used to more efficently sample RAPD marker loci and could potentially reduce the number of primers required for the analysis of genetic relationships among snap bean genotypes by over 60%. The reproducibility of these results has been investigated for the 50 highest ranking primers, for primers ranked by the number of polymorphic RAPD bands scored (Skroch and Nienhuis 1995). On average 4.80 RAPD bands were scored in the original data while 5.42 RAPDs were scored in replicate data. For 39 out of 50 primers the same or a greater number of bands were scored per primer. Eighty percent of the RAPD bands scored in original data were also scored in replicate data.

The statistical independence of RAPDs

The means of the magnitudes of rank correlations for the four different sets of RAPD band pairs and the six possible comparisons among means using t tests ($P = 0.05$) were calculated and are presented in Table 3. The mean correlation for the observed data for bands amplified by the same primer did not differ significantly from the mean computed for bands amplified by different primers. Mean correlations computed for the corresponding randomized data (rows 3 and 4, Table 3) were also not significantly different. The analysis confirms that the independence of RAPDs amplified by the same primer is equivalent to that for RAPDs amplified by different primers. However, the average correlation for observed data was significantly greater than that computed for the corresponding randomized data for both within- and between-primer comparisons of RAPD bands.

The alternative analysis of band differences provides some additional insight into band relationships. The distributions of observed and expected numbers of differences for both among and within primer band comparisons were very similar (Fig. 3a, b). However, based on t-tests the proportions of observations falling into each class were significantly different for observed versus randomized data for 7 of 11 classes for within primer comparisons and 5 of 11 classes for

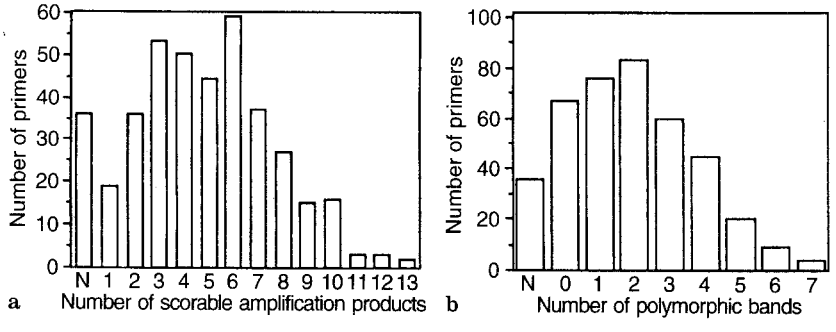


Fig. 2 a Distribution of primers with primers classified by the number of scorable bands, mean ($\pm$ SE) = 5.19 $\pm$ 0.26. Unscorable primers were classified as "N". **b** Distribution of primers with primers classified by number of polymorphic bands scored per primer, mean ($\pm$ SE) = 2.15 $\pm$ 0.17. Unscorable primers classified as "N"

Table 2 Numbers of RAPD polymorphisms among ten snap bean genotypes recorded for 400 RAPD primers

Set[a]	Primer number[b]																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	4	5	1	4	2	2	6	2	5	2	3	0	5	5	1	2	4	3	2	0
B	3	–[c]	2	0	5	–	1	0	0	4	3	3	0	0	3	1	1	0	–	–
F	1	0	1	3	0	5	–	2	4	4	–	1	6	1	–	2	1	–	3	5
G	–	2	5	2	5	3	0	6	2	0	2	0	2	1	2	4	3	1	3	–
H	–	3	3	0	0	3	3	2	2	–	2	4	0	1	4	4	1	3	4	2
I	2	2	4	4	2	4	5	3	2	4	1	0	2	1	4	5	1	4	3	1
J	3	–	2	5	7	3	4	0	2	2	1	1	2	3	2	2	3	1	1	1
M	3	2	2	3	2	1	1	0	4	2	0	6	2	3	3	2	1	0	2	2
O	3	0	2	2	7	0	2	1	0	1	4	3	5	0	3	5	–	1	6	3
P	4	2	1	1	3	1	3	4	2	1	2	–	0	2	2	4	1	1	2	–
Q	3	–	1	2	2	2	1	3	5	1	3	0	4	4	3	2	4	0	–	2
R	0	3	3	5	0	0	5	0	4	0	2	1	3	1	2	0	2	–	2	3
S	–	0	3	0	0	–	2	2	0	2	3	2	1	1	0	0	1	4	0	4
T	2	–	1	2	1	0	3	4	2	–	1	2	1	1	7	1	1	3	–	2
U	5	1	4	–	2	0	0	1	1	1	0	3	4	0	6	3	1	0	6	4
V	1	1	1	2	1	5	–	3	0	6	–	4	–	0	3	0	3	2	1	4
W	4	2	2	2	4	7	1	1	4	0	3	2	3	–	1	2	4	1	3	3
X	3	0	2	4	0	0	2	0	1	–	6	0	0	1	2	–	0	0	–	–
Y	0	2	0	5	3	1	4	1	3	1	2	–	4	1	3	4	4	1	3	4
Z	1	–	0	3	0	1	1	2	–	1	0	1	2	0	2	0	2	2	3	0

[a] Specifies the Operon primer set
[b] Specifies the number of each primer within each set
[c] Indicates poor or no amplification

Table 3 Average magnitudes of Spearman rank correlations for 944 pairwise comparisons of RAPD bands generated by the same or different primers for observed data and data after band scores were randomized among genotypes

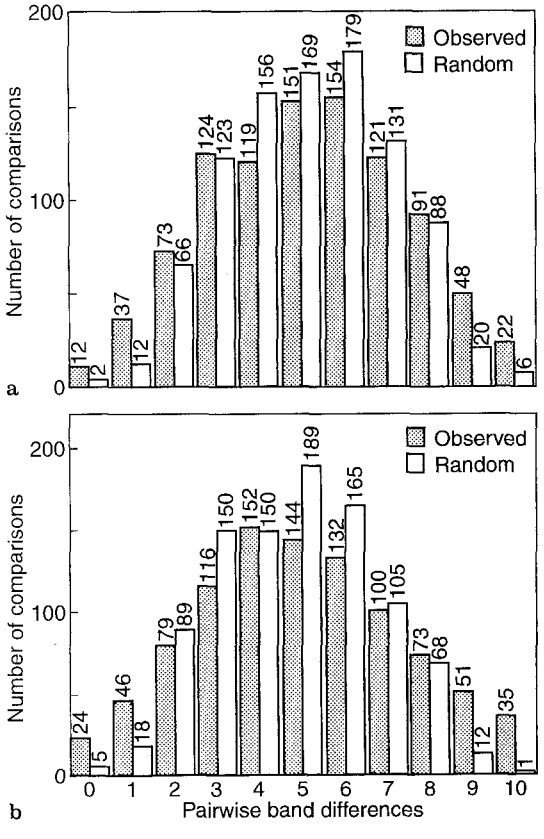
Type of band comparison	Mean correlation ± SE
Within primer, observed	0.376 ± 0.0083 a[a]
Different primers, observed	0.349 ± 0.0075 a
Within primer, random	0.283 ± 0.0056 b
Different primers, random	0.285 ± 0.0057 b

[a] Mean separation by t-test ($P = 0.05$)

bands amplified by different primers. The observed proportions of observations in the extreme classes of 0, 1, 9 and 10 differences were significantly greater than expected for both types of comparisons.

For RAPDs amplified by the same primer, there are several factors which may contribute to a non-random association of markers including the occurrence of co-dominant RAPD markers, the clustering of RAPD markers on the bean genetic map, genetic or linkage disequilibrium among RAPD marker loci, and the anomalous generation of different sized RAPD products with identical distributions among genotypes (Tinker et al. 1993; Huang and Jeang 1994). For RAPDs amplified by different primers, the excess of band pairs that are negatively or positively associated among genotypes cannot be easily explained by the existence of amplification artifacts or co-dominant markers. The need to understand the genetic basis of associations among RAPDs detected by the same or different primers underscores the need for genetic mapping of RAPD polymorphisms used in population genetic studies.

Fig. 3 a Distribution of 952 pairwise band comparisons classified by the number of differences between bands. This distribution is based on 952 band differences randomly selected from the 306, 936 possible pairwise band comparisons. Pairwise band differences were calculated for observed data and data after randomizing band scores among genotypes. **b** Distribution of the 952 possible pairwise band comparisons classified as in **a** for bands amplified by the same primer for both observed and randomized data



"Fingerprinting" RAPDs

In the data reported here, different RAPDs amplified by the same primer rarely showed the same distribution among genotypes; on average, only 24/952 or 2.5% of possible pairs of RAPDs detected by the same primer were identically distributed among genotypes. Depending on the level of random error, these data indicate that individual RAPD markers might be uniquely identified based on the identity of the primer, the approximate fragment size, and the distribution of RAPD amplification among a set of standard genotypes. Unique identification would facilitate communication among researchers, the integration of independently collected data sets, and perhaps the automation of data scoring and analysis.

The uniformity of RAPD amplification potential across genotypes

The generation of similar numbers of amplification products per amplification reaction from genomic templates of very different sizes (Williams et al. 1990) suggests that the mean number of amplification products per reaction is independent of genome size or genome identity. In our study, the hypothesis that band amplification was uniformly distributed among genotypes was tested for the entire data set and for each subset of RAPD bands classified by amplification strength. We hypothesized that genomic template samples of poorer quality would deviate significantly from expectation. Individual chi square tests were non-significant. The total chi square of 2.08 for the entire data set with 8 *df*, was also not significant [$P(>\chi^2) = 0.975$]. In addition, the same chi square tests performed for data subsets defined by band classification into faint, medium, and bold categories were not significant ($P > 0.05$). The consistency of RAPD amplification indicates that the experimental protocol and scoring criteria used in this study resulted in a uniform application of experimental conditions across genotypes.

The relationship between the frequency and intensity of RAPD bands

The classification of polymorphic RAPD bands into faint, medium, and bold categories resulted in 333 faint, 281 medium, and 170 bold bands. The mean ($\pm$ SE) number of occurrences was 4.39 ± 0.16 for RAPDs classified as faint, 4.87 ± 0.18 for RAPDs classified as medium, and 5.17 ± 0.22 for RAPDs classified as bold. Thus, the frequency of amplification of a RAPD band increased with band amplification strength. Based on a t test, the mean for faint bands was significantly lower then that for bold bands but bands classified as medium were not significantly different from the means for either faint or bold categories. These results might be interpreted as an indication that faint bands were underscored compared to bold bands due to poor amplification in some genotypes for some faint bands. According to this interpretation, the rate of underscoring indicated by the difference between the means may average as much as 0.8 bands per ten genotypes or 8% relative to bands in the bold category. This is similar to the results reported by Weeden et al. (1992) in which the error for the underscoring of faint-band amplification among 56 apple genotypes was 5.3% as determined from an analysis of expected versus observed rates of double recombinants. However, in bean (Skroch and Nienhuis 1995), using the same techniques and replicated data, we have observed the scoring error to be 2% or less. The results of these two studies are not necessarily inconsistent. Some scoring errors, such as those caused by competitive inhibition, may be repeatable and may have a greater effect on weakly amplifying compared to strongly amplifying RAPD bands.

RAPD variability among snap bean genotypes

For the average pairwise comparison of genotypes, 201 out of 400 primers, or about 50% of the primers tested, detected at least one RAPD polymorphism. The average number of primers detecting polymorphism per genotype pair in this study is similar to the results of Haley et al. (1994) where 53.6% of 110 primers tested detected polymorphism between a pair of snap bean DNA bulks. This apparently high level of polymorphism found between snap bean genotypes may be surprising given the reported derivation of modern snap bean cultivars from a narrow genetic base (Zaumeyer 1972)). However, consistent with the results reported here, as well as those reported by Haley et al. (1994), the results previously described by Skroch et al. (1992 a, b) have suggested that snap beans have a relatively broad genetic base compared to other bean market classes. On average, 15.8% of all bands scored for a pair of genotypes were polymorphic. Nei's gene diversity averaged over all monomorphic and polymorphic RAPD bands scored was 0.158.

The informativeness of a RAPD is related to its frequency of amplification. Polymorphic RAPDs with no missing data were classified by the number genotypes showing the RAPD. The number of occurrences of individual RAPDs varied from 1 to 9 with a mean ($\pm$ SE) of 4.74 ± 0.10 (Fig. 4 a). Bands were also classified by the number of times each band was present or absent (putative RAPD alleles) depending on which was more common for that band (Fig. 4 b). There is a clear trend with number of RAPDs detected increasing from the most informative with five occurrences to the least informative with nine occurrences. Thus, the most informative RAPDs were also the rarest. The average value of Nei's gene diversity for polymorphic RAPD bands was 0.388. Thus, on average, a RAPD marker detected in this study will segregate in only 38.8% of all crosses among these ten genotypes.

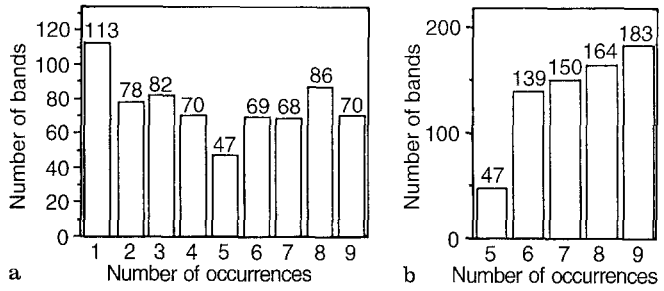
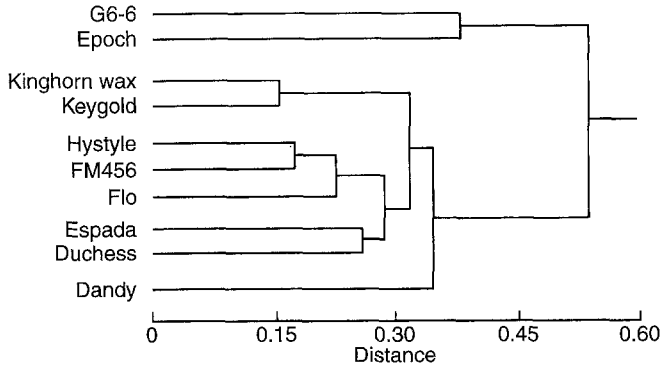


Fig. 4 a Distribution of 683 RAPD bands classified by the number of occurrences of amplification among the ten genotypes studied, mean ($\pm$ SE) = 4.74 $\pm$ 0.10. Only bands with no missing data were included. **b** Distribution of 683 RAPD bands classified by the number of occurrences of the most common RAPD "allele", mean ($\pm$ SE) = 7.43 $\pm$ 0.05

RAPD derived genetic relationships among snap bean genotypes

Genetic distances varied from a maximum of 0.65 between G6.6 and Keygold to a minimum of 0.16 between Keygold and Kinghorn wax with an average of 0.39. The relationships among the different genotypes in this study, based on the genetic distance matrix and the UPGMA clustering algorithm, are presented in Fig. 5. The first major split in the dendogram shows two distinct groups; Epoch and the U. W. breeding line G6–6 are in one cluster while eight other genotypes are in a second group. Two centers of diversity, meso-american and andean, have been established for the common bean germplasm (Gepts 1990). The pedigree of G6–6 contains a substantial contribution from dry bean land races which are of meso-american origin. However, based on Phaseolin protein patterns snap beans appear to be of andean origin (Brown et al. 1982; Gepts et al. 1986). Thus, it is not surprising that G6–6 is relatively unrelated to most of the cultivars employed in the present study. The clustering of Epoch with G6–6 suggests that Epoch also contains meso-american derived germplasm in its pedigree. Within the group containing the eight other cultivars there are three more or less distinct groups: one containing the two wax bean cultivars, one containing five processing green podded snap beans, and one containing the single cultivar Dandy. The distinction between wax beans and green beans is clear. Based on seed-company reports Dandy is also phenotypically distinct in having the smallest pods in this group. The reproducibility of RAPD based estimates of genetic distance across independent sets of RAPD bands is demonstrated for these genotypes by Skroch and Nienhuis (1995).

Fig. 5 Dendogram based on UPGMA analysis of the genetic distance matrix, showing the relationships among the ten genotypes included in this study. For each pairwise comparison of genotypes, genetic distance was computed as the proportion of RAPD bands which were polymorphic for that comparison



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